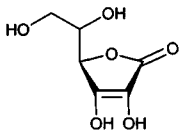
Ascorbic acid



Molecular formula: $C_6H_8O_6$
Molecular weight: 176.13
CAS Registry No.: 50-81-7
Merck Index: 867

SAMPLE

Matrix: beverages, vegetables
Sample preparation: Blend 5 g food with 25 mL 300 mM trichloroacetic acid for 1 min, make up to 50 mL with 300 mM trichloroacetic acid. Dilute 5 g beverage to 50 mL with 300 mM trichloroacetic acid. Filter (paper) these solutions, dilute with 300 mM trichloroacetic acid to a ascorbic acidoncentration of 1-40 μg/mL. Remove a 3 mL aliquot ,and add it to 400 μL 4.5 M pH 6.2 sodium acetate buffer, add an ascorbate oxidase spatula (Boehringer Mannheim), heat at 37° for 2 min, mix, heat at 37° for 3 min, remove the spatula (?), add 500 μL 0.1% o-phenylenediamine (freshly prepared), mix, heat at 37° in the dark for 30 min, inject a 30 μL aliquot.

HPLC VARIABLES

Guard column: 20 mm long RP-18 (Bischoff)
Column: 125 × 4.6 3 μm ODS-Hypersil
Mobile phase: MeOH:80 mM KH_2PO_4 20:80, pH 7.8
Flow rate: 1
Injection volume: 30
Detector: F ex 365 (filter) em 418 (filter)

CHROMATOGRAM

Retention time: 8 (ascorbic acid), 10 (isoascorbic acid)
Limit of detection: 200 ng/g

KEY WORDS

derivatization; protect from light; avocado; brussels sprouts; cabbage; cauliflower; kale; lemon juice; lettuce; orange juice; paprika; parsley; peas

REFERENCE

Speek,A.J.; Schrijver,J.; Schreuers,W.H.P. Fluorometric determination of total vitamin C and total isovitamin C in foodstuffs and beverages by high-performance liquid chromatography with precolumn derivatization, *J.Agric.Food Chem.*, **1984**, *32*, 352–355.

SAMPLE

Matrix: beverages, vegetables
Sample preparation: 500 μL Juice or homogenized vegetables + 5 mg pyrogallol + 10 mL 100 mM citric acid, vortex under nitrogen for 1 min, add an equal volume of dichloromethane, vortex for 1 min, centrifuge at 4° at 1200 g for 10 min, repeat dichloromethane wash (if necessary to remove excess fat). Filter (0.45 μm) the aqueous layer, pass a 2 mL aliquot through a conditioned Sep-Pak C18 (?) SPE cartridge, inject an aliquot of the eluate.

HPLC VARIABLES

Column: 150 × 5 DA-X8-11 anion-exchange resin (Dionex)
Mobile phase: 100 mM pH 3.8 Citrate buffer containing 10 mM NaCl and 5 mM EDTA
Flow rate: 0.5
Injection volume: 100
Detector: F ex Corning 7-60 filter em Wratten 2-E following post-column reaction. The column effluent mixed with the oxidizer pumped at 0.5 mL/min and this mixture flowed

through a 32 cm × 0.25 mm ID stainless-steel coil. The effluent from this coil mixed with the reagent pumped at 0.5 mL/min and this mixture flowed through a 45.7 m × 0.25 mm ID stainless-steel coil at 70° then a 1.5 m × 0.25 mm ID stainless-steel coil at 20° to the detector. (Oxidizer was 2.5 mM mercuric chloride or copper sulfate in mobile phase. Reagent was 3.1 mM o-phenylenediamine in mobile phase.)

CHROMATOGRAM
Retention time: 23
Limit of detection: 20 ng

OTHER SUBSTANCES
Extracted: dehydroascorbic acid

KEY WORDS
post-column reaction; bean sprouts; beets; broccoli; grape juice; orange juice; potatoes; tomatoes; SPE

REFERENCE
Vanderslice,J.T.; Higgs,D.J. HPLC analysis with fluorometric detection of vitamin C in food samples, *J.Chromatogr.Sci.*, **1984**, *22*, 485–489.

SAMPLE
Matrix: blood
Sample preparation: Dilute an aliquot of plasma or serum with an equal volume of 10% metaphosphoric acid, add IS to a final concentration of 4.5 μg/mL, centrifuge at 3300 g for 10 min, filter the supernatant (0.22 μm), inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS C18
Mobile phase: 50 mM pH 4.5 KH_2PO_4 containing 5 mM cetyltrimethylammonium bromide
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.7
Internal standard: 4-hydroxyacetanilide (13.9)
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: dehydroascorbic acid

KEY WORDS
plasma; serum

REFERENCE
Esteve,M.J.; Farré,R.; Frigola,A.; Garcia-Cantabella,J.M. Determination of ascorbic and dehydroascorbic acids in blood plasma and serum by liquid chromatography, *J.Chromatogr.B*, **1997**, *688*, 345–349.

SAMPLE
Matrix: blood
Sample preparation: Collect 5 mL whole blood in a tube with 100 μL glutathione solution. Remove a 1 mL aliquot and add it to 4 mL 300 mM trichloroacetic acid, vortex thoroughly for 10 min, let stand in the dark at 4° for 10 min, mix, let stand in the dark at 4° for 10 min, centrifuge at 4° at 2000 g for 10 min. Remove a 1.5 mL aliquot of the supernatant and add it to 200 μL 4.5 M pH 6.2 sodium acetate buffer, add an ascorbate oxidase spatula (Boehringer Mannheim), heat at 37° for 2 min, mix, heat at 37° for 3 min, remove the

spatula, add 250 μL 0.1% o-phenylenediamine (freshly prepared), mix, heat at 37° in the dark for 30 min, inject a 20 μL aliquot. (Prepare glutathione solution by dissolving 1.5 g glutathione in 25 mL water, adjust pH to 6.5 with 2 M NaOH, add 2.25 g ethyleneglycolbis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA), adjust pH to 6.5 with 10 M NaOH.)

HPLC VARIABLES
Column: 80 × 4.6 3 μm ODS-Hypersil
Mobile phase: MeOH:80 mM KH_2PO_4 20:80, pH 7.8
Flow rate: 1
Injection volume: 20
Detector: F ex 365 (filter) em 418 (filter)

CHROMATOGRAM
Retention time: 3
Limit of detection: 200 nM

KEY WORDS
derivatization; protect from light; whole blood

REFERENCE
Speek,A.J.; Schrijver,J.; Schreurs,W.H. Fluorometric determination of total vitamin C in whole blood by high-performance liquid chromatography with pre-column derivatization, *J.Chromatogr.*, **1984**, *305*, 53–60.

SAMPLE
Matrix: blood
Sample preparation: Dilute plasma with an equal volume of cold 10% metaphosphoric acid containing 0.54 mM disodium EDTA, centrifuge, mix the supernatant with an equal volume of cold 5% metaphosphoric acid containing 0.54 mM disodium EDTA and 10 μg/mL isoascorbic acid, dilute 25 fold with cold 1.04 mM cysteine containing 0.54 mM EDTA, filter (0.2 μm), inject a 50 μL aliquot of the filtrate. (Maintain at 4° during sample preparation and in autosampler.)

HPLC VARIABLES
Guard column: 30 × 4.6 5 μm RP-18 (Brownlee)
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeOH:buffer 7.5:92.5 adjusted to pH 4.75 with glacial acetic acid (Buffer was 40 mM sodium acetate containing 0.54 mM disodium EDTA and 1.5 mM dodecyltrimethylammonium phosphate.) (At the end of each week wash column with 50-100 mL water and 50-100 mL MeOH, store in MeOH.)
Flow rate: 0.8
Injection volume: 50
Detector: E, Bioanalytical systems LC4B, glassy carbon working electrode, stainless steel electrode top, Ag/AgCl reference electrode, +0.5 V

CHROMATOGRAM
Retention time: 14.8
Internal standard: isoascorbic acid (16.1)
Limit of detection: 0.02 ng
Limit of quantitation: 0.2 ng

KEY WORDS
plasma

REFERENCE
Kutnink,M.A.; Hawkes,W.C.; Schaus,E.E.; Omaye,S.T. An internal standard method for the unattended high-performance liquid chromatographic analysis of ascorbic acid in blood components, *Anal. Biochem.*, **1987**, *166*, 424–430.

SAMPLE
Matrix: blood
Sample preparation: 20 μL Plasma + 10 μL 125 μg/mL α-methyl-L-dopa in water, add to a PCPure hydroxyapatite SPE cartridge (Moritex or Koken), elute with buffer, collect 800 μL eluate, inject a 20 μL aliquot. (Buffer was freshly prepared 10 mM pH 6.8 sodium phosphate buffer containing 2.5 mg/mL L-cysteine.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: 100 mM KH_2PO_4 containing 1 mM disodium EDTA, adjusted to pH 3 with phosphoric acid
Flow rate: 0.6
Injection volume: 20
Detector: E, Irica Σ875, 300 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3.5
Internal standard: α-methyl-L-dopa (15)
Limit of detection: 240 ng/mL

KEY WORDS
plasma; SPE

REFERENCE
Iwase,H.; Ono,I. Determination of ascorbic acid in human plasma by high-performance liquid chromatography with electrochemical detection using a hydroxyapatite cartridge for precolumn deproteinization, *J.Chromatogr.B*, **1994**, *655*, 195–200.

SAMPLE
Matrix: blood
Sample preparation: Add one volume of 10% metaphosphoric acid to 3 volumes of plasma, freeze the clear supernatant at -80°, thaw, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 4 μm triacontyl Daltosil 100 C30 (Serva)
Mobile phase: 13.61 g/L KH_2PO_4 adjusted to pH 2.34 with concentrated orthophosphoric acid
Flow rate: 0.5 for 10 min then 1 for 5 min
Injection volume: 10
Detector: UV 250

CHROMATOGRAM
Retention time: 9.9
Limit of detection: 2 ng

KEY WORDS
plasma

REFERENCE
Manoharan,M.; Schwille,P.O. Measurement of ascorbic acid in human plasma and urine by high-performance liquid chromatography. Results in healthy subjects and patients with idiopathic calcium urolithiasis, *J.Chromatogr.B*, **1994**, *654*, 134–139.

SAMPLE
Matrix: blood
Sample preparation: 600 μL Plasma + 600 μL 10% metaphosphoric acid, freeze, store at -80°, allow to thaw at 4° for 1 h, centrifuge at 4° at 1000 g for 10 min, inject a 20 μL aliquot of the supernatant

HPLC VARIABLES
Guard column: 20 × 4 30-40 μm Perisorb pellicular C18 (Anachem)
Column: 250 × 4.6 5 μm Nucleosil ODS
Mobile phase: MeCN:buffer 7.5:92.5, pH 5.5 (Buffer was 25 mM myristyltrimethylammonium bromide containing 50 mM NaOH, 60 mM acetic acid, 100 μg/mL homocysteine, and 200 μg/mL EDTA.)
Flow rate: 0.55
Injection volume: 20
Detector: UV 262

CHROMATOGRAM
Retention time: 5.32

OTHER SUBSTANCES
Extracted: uric acid

KEY WORDS
plasma

REFERENCE
Ross,M.A. Determination of ascorbic acid and uric acid in plasma by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *657*, 197–200.

SAMPLE
Matrix: blood
Sample preparation: 30 μL Plasma + 60 μL stabilizing solution, mix, let stand on ice for 10-15 min, centrifuge at 4° at 12000 g for 5 min, inject a 1-5 μL aliquot of the supernatant. (Stabilizing solution was MeOH:water 90:10 saturated with EDTA.)

HPLC VARIABLES
Column: 250 × 4.5 QC Pack C18 (IRICA) or 250 × 4.5 TSK gel ODS 120A (Toyo Soda)
Mobile phase: MeOH:water 20:80 containing 50 mM sodium phosphate, 50 mM sodium acetate, 189 μm dodecyltrimethylammonium chloride, 36.6 μm tetraoctylammonium bromide, 0.2 mM EDTA, pH adjusted to 4.8 with phosphoric acid (Dissolve dodecyltrimethylammonium chloride in MeOH first.)
Flow rate: 1
Injection volume: 1-5
Detector: E, IRICA Sigma 875, +350 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3
Limit of detection: 0.1 ng

KEY WORDS
plasma

REFERENCE
Umegaki,K.; Inoue,K.; Takeuchi,N.; Higuchi,M. Improved method for the analysis of ascorbic acid in plasma by high-performance liquid chromatography with electrochemical detection, *J.Nutr. Sci.Vitaminol.(Tokyo)*, **1994**, *40*, 73–79.

SAMPLE

Matrix: blood, food, formula, perfusate

Sample preparation: Serum, perfusate. Vortex 1.5 mL plasma or perfusate with 160 μL 400 mg/mL metaphosphoric acid in water for 15 s, add 400 μL MeCN vortex for 15 s, centrifuge at 4° at 1000 g for 30 min, inject an aliquot (Clin.Chem. 1988, 34, 2217). Infant formula. Mix 5 g formula with 40 mL 50 mM monobasic potassium phosphate in water containing 1 g/L dithiothreitol, allow to dissolve. Add 10 mL 500 g/L metaphosphoric acid in water and 20 mL MeCN, mix, centrifuge at 1000 g for 15 min at 5°. Remove a 4 mL aliquot of the clear lower layer, dilute with 50 mM monobasic potassium phosphate in water containing 1 g/L dithiothreitol, inject an aliquot. Food (for total ascorbic acid). Suspend 1 g pureed food in 5 mL water, vortex for 15 s, add 1 mL 500 mM dibasic potassium phosphate in water containing 100 g/L dithiothreitol, vortex for 15 s, let stand at room temperature for 30 min, add 1 mL 400 g/L metaphosphoric acid in water, add 2 mL MeCN, vortex for 15 s, centrifuge at 1000 g for 30 min at 5°, inject an aliquot of the clear lower layer.

HPLC VARIABLES

Column: 250 × 4.6 Capcell Pak NH2 (Shiseido, Japan)

Mobile phase: MeCN:buffer 80:20 (Prepare mobile phase as follows. Dissolve 680 mg monobasic potassium phosphate in 200 mL water, add 800 mL MeCN and 7.5 mL concentrated phosphoric acid.)

Column temperature: 40

Flow rate: 1

Detector: E, Model 400 (EG & G, Princeton Applied Research, USA), 700 mV

CHROMATOGRAM

Retention time: 4.2

OTHER SUBSTANCES

Extracted: dithiothreitol, uric acid

KEY WORDS

human; serum; rat

REFERENCE

Margolis,S.A.; Schapira,R.M. Liquid chromatographic measurement of L-ascorbic acid and D-ascorbic acid in biological samples, *J.Chromatogr.B*, **1997**, *690*, 25–33.

SAMPLE

Matrix: blood, formulations

Sample preparation: Tablets. Powder tablets, dissolve in water, inject a 10 μL aliquot. Injections. Dilute with water, inject a 10 μL aliquot. Plasma. Condition a Lichrolut RP-18 (Merck) SPE cartridge with 3 mL MeOH and 3 mL water. 40 μL Plasma + 80 μL MeCN, mix for 2 min, add 100 μL water, centrifuge at 3500 rpm for 15 min, evaporate the supernatant under nitrogen at 45° to remove the organic solvents, add slowly to the SPE cartridge, collect the eluate. Evaporate to dryness under a stream of nitrogen at 45°. Reconstitute the residue with 500 μL MeOH containing 4.2 μg/mL IS. Inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4 5 μm Lichrosorb RP-18

Mobile phase: Gradient. A was MeOH. B was 50 mM ammonium acetate. A:B from 5:95 to 15:85 over 6 min, to 30:70 over 7 min, maintain at 30:70 over 7 min

Flow rate: 1

Injection volume: 10

Detector: UV 270

CHROMATOGRAM
Retention time: 1.89
Internal standard: xanthine (4.56)
Limit of detection: 2.5 ng

OTHER SUBSTANCES
Extracted: folic acid, niacin, niacinamide, riboflavin, vitamin B12

KEY WORDS
plasma; SPE; tablets; injections

REFERENCE
Papadoyannis,I.N.; Tsioni,G.K.; Samanidou,V.F. Simultaneous determination of nine water and fat soluble vitamins after SPE separation and RP-HPLC analysis in pharmaceutical preparations and biological fluids, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 3203–3231.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma. 200 μL Plasma + 200 μL 10% metaphosphoric acid, mix, centrifuge at 0° at 6000 g for 10 min, filter (Millipore Molcut II), inject a 10 μL aliquot of the filtrate. Liver. Homogenize 500 mg liver with 10 mL 5% metaphosphoric acid, mix, centrifuge at 0° at 6000 g for 10 min, filter (Millipore Molcut II), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 300 × 7.9 10 μm Shimpack SCR-101H (Shimadzu)
Mobile phase: 5 mM oxalic acid
Column temperature: 30
Flow rate: 0.8
Injection volume: 10
Detector: UV 300 following post-column reaction. The column effluent mixed with 100 mM sodium borohydride in 100 mM NaOH pumped at 0.6 mL/min, the mixture flowed through a "j" type reaction coil (Shimadzu) maintained at 30° to the detector.

CHROMATOGRAM
Retention time: 16.8
Limit of detection: 100 ng/mL

OTHER SUBSTANCES
Extracted: dehydroascorbic acid

KEY WORDS
fish; carp; yellowtail; liver; plasma; post-column reaction

REFERENCE
Ito,T.; Murata,H.; Yasui,Y.; Matsui,M.; Sakai,T.; Yamauchi,K. Simultaneous determination of ascorbic acid and dehydroascorbic acid in fish tissues by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *667*, 355–357.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. 1 mL Plasma + 3 mL 10 g/L metaphosphoric acid, mix, centrifuge at 1500 g. Mix a 500 μL aliquot of the supernatant with 50 μL IS solution, inject a 20 μL aliquot. Urine. Dilute 1 mL urine with 3 mL 10 g/L metaphosphoric acid. Mix a 500 μL aliquot with 50 μL IS solution, inject a 20 μL aliquot. (IS solution was 100 mg/L D-isoascorbic acid in 10 g/L metaphosphoric acid containing 2 mM EDTA (nitrogen-saturated).)

HPLC VARIABLES

Guard column: 70 × 2 10 μm PRP-1 (Hamilton)
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: 0.15 mM Hexadecyltrimethylammonium bromide containing 0.5 mM sodium acetate and 0.15 mM disodium EDTA, adjusted to pH 4.0 with acetic acid. (Condition system with twenty 20 μL aliquots of 10 g/L metaphosphoric acid.)
Flow rate: 0.5
Injection volume: 20
Detector: F ex 365 em 440 following post-column reaction. The column effluent mixed with the reagent pumped at 1.5 mL/min and flowed through a 20 m × 0.55 mm ID PTFE coil at 65° then a 1.5 m × 0.55 mm ID PTFE coil at 20° to the detector. (Reagent was 2.5 mM cupric sulfate containing 52 mM citric acid and 0.5 mM 4,5-dimethyl-o-phenylenediamine dihydrochloride, adjusted to pH 4.1 with saturated NaOH. Prepare fresh each day. Prepare 4,5-dimethyl-o-phenylenediamine dihydrochloride by dissolving 4,5-dimethyl-o-phenylenediamine in a minimum volume of diethyl ether, pass anhydrous hydrogen chloride through the solution for 20 min, precipitate the salt with diethyl ether. Wash the salt three times with diethyl ether.)

CHROMATOGRAM

Retention time: 15.5
Internal standard: isoascorbic acid (19.9)
Limit of detection: 10 ng

OTHER SUBSTANCES

Extracted: dehydroascorbic acid, dehydroisoascorbic acid

KEY WORDS

post-column reaction; plasma

REFERENCE

Lopez-Anaya,A.; Mayersohn,M. Ascorbic and dehydroascorbic acids simultaneously quantified in biological fluids by liquid chromatography with fluorescence detection, and comparison with a colorimetric assay, *Clin.Chem.*, **1987**, *33*, 1874–1878.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 249.9

CHROMATOGRAM
Retention time: 2.928

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: food
Sample preparation: Blend food with 5% metaphosphoric acid containing L-cysteine, centrifuge at 4° at 57600 g for 10 min, filter (0.45 μm), immediately inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 250 × 4.6 C18 (Beckman)
Mobile phase: MeOH:water 75:25
Flow rate: 0.5
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 10
Limit of detection: 1.2 μg/mL

KEY WORDS
comparison with capillary electrophoresis; fruit; vegetables

REFERENCE
Choi,O.-K.; Jo,J.-S. Determination of L-ascorbic acid in foods by capillary zone electrophoresis, *J.Chromatogr.A*, **1997**, *781*, 435–443.

SAMPLE
Matrix: formulations
Sample preparation: Tablets without iron. Grind 5 tablets to a fine powder, add 10 mL monothioglycerol and 800 mL buffer, sonicate for 30 min, add 150 mL MeOH, make up to 1 L with buffer, filter (GF/C paper), discard first few mL, remove a 10 mL aliquot, make up to 25 mL with mobile phase, inject an aliquot. Tablets with dioctyl sodium sulfosuccinate. Grind 5 tablets to a fine powder, add 10 mL 2-monothioglycerol and 1 g barium chloride, make up to 1 L with buffer, stir vigorously for 30 min, filter (GF/C paper), discard first few mL, inject an aliquot. Capsules with iron. Contents of one capsule + 5 mL 2-monothioglycerol + 2 mL glacial acetic acid + 75 mL buffer, sonicate for 5 min, make up to 100 mL with buffer, stir vigorously for 30 min, filter (GF/C paper), add 300 mg cupferron, stir for 10 min, let stand for 1 h at room temperature, filter (GF/C paper), let stand for 30 min, filter again (if necessary), discard first few mL, inject an aliquot. (Buffer was 48 mL glacial acetic acid and 10 mL triethylamine in 1 L water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine, make up to 1.7 L with water.)

HPLC VARIABLES
Column: 100 × 8 Radial Pak A C18 (Waters)
Mobile phase: MeOH:buffer 15:85 (Buffer was 2.20 g sodium heptanesulfonate, 100 mg EDTA, 48 mL glacial acetic acid, and 10 mL triethylamine made up to 1.7 L with water, adjust pH to 3.6 ± 0.05 with acetic acid or triethylamine.)
Flow rate: 2

Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 1.3

OTHER SUBSTANCES
Simultaneous: niacinamide (UV 280), thiamine (UV 280), riboflavin (UV 280), pyridoxine (UV 280)

KEY WORDS
multi-vitamin; protect from light; tablets; capsules

REFERENCE
Lam,F.-L.; Holcomb,I.J.; Fusari,S.A. Liquid chromatographic assay of ascorbic acid, niacinamide, pyridoxine, thiamine, and riboflavin in multivitamin-mineral preparations, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 1007–1011.

SAMPLE
Matrix: formulations
Sample preparation: Pulverize tablets and weigh out 1 g, add 1 mL formic acid, add 25 mL MeOH, shake mechanically for 10 min, make up to 50 mL with methanol. Remove 10 mL and centrifuge. 5 mL Supernatant + 5 mL 0.0025% p-hydroxybenzoic acid in MeOH:water 20:80, make up to 25 mL with water, inject an aliquot. (Analyze within 1 h.)

HPLC VARIABLES
Column: 250 × 4.6 LiChrosorb RP8
Mobile phase: MeOH:200 mM pH 3.5 phosphate buffer:water 20:10:70
Flow rate: 1
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 3
Internal standard: p-hydroxybenzoic acid (18)

OTHER SUBSTANCES
Simultaneous: aspirin, p-aminophenol, 3-O-acetylascorbic acid, 2-O-acetylascorbic acid, saccharin, acetaminophen, O-acetyl-p-aminophenol, salicylic acid (UV 280), diacetyl-p-aminophenol (UV 280)

KEY WORDS
tablets

REFERENCE
Thomis,R.; Roets,E.; Hoogmartens,J. Analysis of tablets containing aspirin, acetaminophen, and ascorbic acid by high-performance liquid chromatography, *J.Pharm.Sci.*, **1984**, *73*, 1830–1833.

SAMPLE
Matrix: formulations
Sample preparation: Dilute injections with water, inject a 50 μL aliquot. Dissolve tablets or capsule contents in water (warm if necessary), filter (0.5 μm PTFE), inject a 50 μL aliquot of the filtrate. (Dissolve tablets or other formulations containing proteinaceous material in water at 60°, add 5% trichloroacetic acid (to pH 4.4), filter, inject a 50 μL aliquot.)

HPLC VARIABLES
Guard column: pellicular Corasil
Column: 10 μm μBondapak C18
Mobile phase: Gradient. A was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 170 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 2.5 with 1 M KOH, make up to 1 L. B was prepared by dissolving 1 g sodium dioctylsulfosuccinate in 450 mL MeOH, add 10 mL concentrated formic acid, make up to 800 mL with water, adjust pH to 4.6, make up to 1 L. A:B from 100:0 to 0:100 over 25 min (concave curve 9), maintain at 0:100 for 3 min, return to initial conditions over 2 min.
Flow rate: 1.5
Injection volume: 50
Detector: UV 280

CHROMATOGRAM
Retention time: 2

OTHER SUBSTANCES
Simultaneous: folic acid, niacin (UV 254), niacinamide (UV 254), pyridoxamine, thiamine (UV 254), riboflavin (UV 254), pyridoxine

KEY WORDS
injections; capsules; tablets

REFERENCE
Woollard,D.C. New ion-pair reagent for the high-performance liquid chromatographic separation of B-group vitamins in pharmaceuticals, *J.Chromatogr.*, **1984**, *301*, 470–476.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 500 mg ground tablets, extract with water, make up to 50 or 100 mL with water, filter, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 10 C18
Mobile phase: MeOH:1% acetic acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 270

CHROMATOGRAM
Retention time: 2.8

OTHER SUBSTANCES
Simultaneous: menadione hydrogen sulfite, niacinamide, riboflavin, thiamine
Interfering: pyridoxine

KEY WORDS
tablets; multi-vitamin

REFERENCE
Sadlej-Sosnowska,N.; Blitek,D.; Wilczynska-Wojtulewicz,I. Determination of menadione sodium hydrogen sulphite and nicotinamide in multivitamin formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1986**, *357*, 227–232.

SAMPLE
Matrix: formulations

HPLC VARIABLES
Column: 100 × 4 3 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:water adjusted to pH 2.1 from 0.3:99.7 to 25:75 over 11 min
Flow rate: 0.5
Detector: UV 220

CHROMATOGRAM
Retention time: 2.3

OTHER SUBSTANCES
Simultaneous: biotin, caffeine, citric acid, folic acid, niacinamide, niacin, pantothenic acid, riboflavin, saccharin, thiamine, pyridoxine, vitamin B12

KEY WORDS
tablets

REFERENCE
Hewlett Packard Leaflet 12-5091-7351 EUS, 1993, **1993**,

SAMPLE
Matrix: fruit, vegetables
Sample preparation: Juices. Dilute 2 mL fruit juice to 50 mL with 0.05% disodium EDTA in 100 mM sulfuric acid, filter (Whatman No. 1 paper), inject a 10 μL aliquot of the filtrate. Fruit, vegetables. Blend (Waring) fruit or vegetable with 0.05% disodium EDTA in 100 mM sulfuric acid for 3 min, centrifuge at 15000 rpm for 10 min. Remove the supernatant and make it up to 10 mL with 0.05% disodium EDTA in 100 mM sulfuric acid, inject a 10 μL aliquot.

HPLC VARIABLES
Guard column: MicroGuard ion exclusion cartridge (Bio-Rad)
Column: 300 × 7.8 Aminex HPX-87 (Bio-Rad)
Mobile phase: 4.5 mM Sulfuric acid
Flow rate: 0.5
Injection volume: 10
Detector: UV 245

CHROMATOGRAM
Retention time: 12.9
Limit of quantitation: 50 ng

KEY WORDS
juice; orange; lemon; grapefruit; pineapple; tomatoes; peas; potatoes; strawberries; green peppers

REFERENCE
Ashoor,S.H.; Monte,W.C.; Welty,J. Liquid chromatographic determination of ascorbic acid in foods, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 78–80.

SAMPLE
Matrix: juice
Sample preparation: 15 g Orange juice + 5 mL 12.5% trichloroacetic acid, centrifuge, filter, inject a 10 μL aliquot of the filtrate. (To determine ascorbic acid and dehydroascorbic acid (by reducing dehydroascorbic acid to ascorbic acid) dilute filtrate with water to an ascorbic acid concentration of 10-100 μg/mL adjust pH to 7.0. Remove a 500 μL aliquot and add it to 2 mL 0.8% DL-homocysteine, let stand for 15 min, filter, inject a 10 μL aliquot.)

HPLC VARIABLES
Column: 250 × 4.6 5 μm Erbasil NH2
Mobile phase: MeOH:0.25% pH 3.5 KH_2PO_4 50:50
Flow rate: 0.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8.7

KEY WORDS
orange juice; comparison with capillary electrophoresis

REFERENCE
Chiari,M.; Nesi,M.; Carrea,G.; Righetti,P.G. Determination of total vitamin C in fruits by capillary zone electrophoresis, *J.Chromatogr.*, **1993**, *645*, 197–200.

SAMPLE
Matrix: juice
Sample preparation: Dilute with water to a ascorbic acid concentration of 10 μg/mL, filter (0.45 μm), remove a 20 μL aliquot of the filtrate and add it to 10 μL 125 μg/mL α-methyl-L-dopa in water and 800 μL 2% metaphosphoric acid, inject a 20 μL aliquot. (To measure total ascorbic acid content after reduction of dehydroascorbic acid dilute juice with water to a ascorbic acid concentration of 10 μg/mL, filter (0.45 μm), remove a 20 μL aliquot of the filtrate and add it to 10 μL 125 μg/mL α-methyl-L-dopa in water and 800 μL buffer, let stand for 15 min, inject a 20 μL aliquot. (Buffer was freshly prepared 10 mM pH 6.8 sodium phosphate buffer containing 2.5 mg/mL L-cysteine.))

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: 100 mM KH_2PO_4 containing 1 mM disodium EDTA, adjusted to pH 3 with phosphoric acid
Flow rate: 0.6
Injection volume: 20
Detector: E, Irica Σ875, 300 mV, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 3.5
Internal standard: α-methyl-L-dopa (15)
Limit of detection: 0.15 ng

KEY WORDS
lemon; grape; orange; apple; tea

REFERENCE
Iwase,H.; Ono,I. Determination of ascorbic acid and dehydroascorbic acid in juices by high-performance liquid chromatography with electrochemical detection using L-cysteine as precolumn reductant, *J.Chromatogr.A*, **1993**, *654*, 215–220.

SAMPLE
Matrix: plants
Sample preparation: Pulverize 200 mg plant tissue in liquid nitrogen, extract twice with a total volume of 3% metaphosphoric acid containing 1 mM EDTA, centrifuge, pass through a conditioned C18 SPE cartridge (Millipore), inject an aliquot of the last 500 μL of the eluate.; SPE

HPLC VARIABLES
Column: 250 × 4.6 3 μm C18 (Bio-Rad)

Mobile phase: pH 2.5 Phosphoric acid containing 0.1 mM EDTA
Flow rate: 0.8
Injection volume: 20
Detector: UV 248

CHROMATOGRAM
Retention time: 3.2

KEY WORDS
comparison with capillary electrophoresis; SPE

REFERENCE
Davey,M.W.; Bauw,G.; Montagu,M.V. Analysis of ascorbate in plant tissues by high-performance capillary zone electrophoresis, *Anal.Biochem.*, **1996**, *239*, 8–19.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Capcell Pak NH_2 (Shiseido, Japan)
Mobile phase: MeCN:buffer 80:20 (Prepare mobile phase as follows. Mix 680 mg monobasic potassium phosphate, 7.5 mL concentrated phosphoric acid with 200 mL water, add 800 mL MeCN.)
Column temperature: 40
Flow rate: 1.0
Detector: E, Model 400 (EG & G, Princeton Applied Research, Princeton, NJ), 700 mV

CHROMATOGRAM
Retention time: 4.4

OTHER SUBSTANCES
Simultaneous: dithiothreitol, isoascorbic acid, metaphosphoric acid, uric acid,

REFERENCE
Margolis,S.A.; Duewer,D.L. Measurement of ascorbic acid in human plasma and serum: stability, intralaboratory repeatability, and interlaboratory reproducibility, *Clin.Chem.*, **1996**, *42*, 1257–1262.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV 261

CHROMATOGRAM
Retention time: 3

OTHER SUBSTANCES
Simultaneous: salicylic acid, benzoic acid, quinine, dihydroquinine

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 33 × 4.6 3 μm Supelcosil LC-8-DB
Mobile phase: MeOH:buffer 15:85 (Buffer was 4.3 mM sodium hexanesulfonate containing 0.1% triethylamine, adjusted to pH 2.8 with phosphoric acid.)
Column temperature: 35
Flow rate: 1
Detector: UV 200

CHROMATOGRAM

Retention time: 0.5

OTHER SUBSTANCES

Simultaneous: niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, niacinamide

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 780.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 100 × 4.6 Spheri-5 RP-8
Mobile phase: Gradient. A was 100 mM pH 4.7 acetate buffer. B was MeCN:100 mM pH 4.7 acetate buffer 25:75.
Column temperature: 26
Flow rate: 4
Detector: UV 254

CHROMATOGRAM

Retention time: 0.6

OTHER SUBSTANCES

Simultaneous: niacin, niacinamide, pyridoxine, riboflavin, thiamine

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 7.21.

SAMPLE

Matrix: tissue
Sample preparation: Deproteinize heart tissue with ice-cold 600 mM perchloric acid, neutralize, centrifuge at 4° at 26890 g for 15 min, filter (0.45 μm) the supernatant, inject a 20 μL aliquot of the filtrate.

HPLC VARIABLES

Guard column: 20 × 4.6 3 μm LC-18-T (Supelco)
Column: 150 × 4.6 3 μm LC-18-T (Supelco)
Mobile phase: Gradient. A was MeOH:10 mM KH_2PO_4 1:99, pH 7.0. B was MeOH:100 mM KH_2PO_4 containing 2.8 mM tetrabutylammonium hydroxide 30:70, pH 5.5. A:B 100:0 for

12 min, to 60:40 over 2 min, to 56:44 over 11 min, to 0:100 over 10 min, maintain at 0:100 for 5 min, re-equilibrate at initial conditions for 5 min.
Flow rate: 1.2
Injection volume: 20
Detector: UV 266

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Extracted: adenosine, adenosine triphosphate

KEY WORDS
rat; heart

REFERENCE
Lazzarino,G.; Di Pierro,D.; Tavazzi,B.; Cerroni,L.; Giardina,B. Simultaneous separation of malondialdehyde, ascorbic acid, and adenine nucleotide derivatives from biological samples by ion-pairing high-performance liquid chromatography, *Anal.Biochem.*, **1991**, *197*, 191–196.

SAMPLE
Matrix: urine
Sample preparation: Prepare a column by suspending 200-400 mesh Dowex 50W-X8 resin in water and pouring it into a 100 × 7 column, allow to settle, wash with 10 mL 2 M HCl, wash with water until the washings are neutral. Mix urine with an equal volume of 5% metaphosphoric acid containing 0.5% β-thiodiglycol. Add a 1 mL aliquot to the column, wash with 3.95 mL 2 mM tartaric acid containing 0.05% β-thiodiglycol, collect all the effluent from the column in a tube containing 50 μL 5% disodium EDTA cooled in ice, filter (0.45 μm) the eluate, inject a 50-250 μL aliquot.

HPLC VARIABLES
Column: two 50 × 7.6 Asahipak GS-320 hydrophilic gel columns in series
Mobile phase: 2.25 g/L Tartaric acid containing 0.75 g/L disodium EDTA and 0.5 g/L β-thiodiglycol, adjusted to pH 3.00-3.03 with 4 M NaOH
Column temperature: 30
Flow rate: 1
Injection volume: 50-250
Detector: F ex 325 em 400 following post-column reaction. The column effluent mixed with 20 mM benzamidine hydrochloride pumped at 0.36 mL/min and with 750 mM pH 10 potassium borate buffer containing 200 mM potassium sulfite pumped at 0.36 mL/min and the mixture flowed through a 50 m × 0.5 mm ID PTFE tube at 90° to the detector.

CHROMATOGRAM
Retention time: 48

OTHER SUBSTANCES
Extracted: isoascorbic acid
Simultaneous: dehydroascorbic acid, diketogluconic acid, diketogulonic acid

KEY WORDS
post-column reaction; SPE

REFERENCE
Seki,T.; Yamaguchi,Y.; Noguchi,K.; Yanagihara,Y. Determination of ascorbic acid in human urine by high-performance liquid chromatography coupled with fluorimetry after post-column derivatization with benzamidine, *J.Chromatogr.*, **1987**, *385*, 287–291.

SAMPLE
Matrix: urine
Sample preparation: Filter (paper), dilute 6 times with 0.05% metaphosphoric acid, inject an aliquot. Alternatively, dilute with an equal volume of 200 mM dithiothreitol, let stand at room temperature for 30 min, dilute three fold with 0.05% metaphosphoric acid, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm Ultrasphere
Mobile phase: 13.61 g/L KH_2PO_4 adjusted to pH 2.34 with concentrated orthophosphoric acid
Flow rate: 0.5 for 10 min then 1 for 5 min
Injection volume: 10
Detector: UV 250

CHROMATOGRAM
Retention time: 8
Limit of detection: 1.5 ng

REFERENCE
Manoharan,M.; Schwille,P.O. Measurement of ascorbic acid in human plasma and urine by high-performance liquid chromatography. Results in healthy subjects and patients with idiopathic calcium urolithiasis, *J.Chromatogr.B*, **1994**, *654*, 134–139.

SAMPLE
Matrix: wine
Sample preparation: Adjust pH of wine to 7-8 with potassium bicarbonate. Remove a 1 mL aliquot and add it to 1 mL 170 mM phenacyl bromide in acetone, add 1 mL 17 mM 18-crown-6 in acetone, add 1 mL acetone, heat in a boiling water bath for 75 min, cool, inject a 10 μL aliquot. (Recrystallize phenacyl bromide from n-heptane.)

HPLC VARIABLES
Guard column: 37-50 μm Bondapak C18/Corasil
Column: 250 × 4 7 μm RP-18 (Merck)
Mobile phase: Gradient. MeOH:water from 35:65 to 85:15 over 20 min.
Flow rate: 2
Injection volume: 10
Detector: UV 254

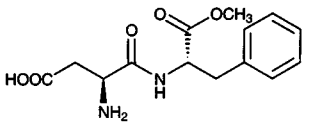
CHROMATOGRAM
Retention time: 11.1

OTHER SUBSTANCES
Extracted: acetic acid, anisic acid, benzilic acid, benzoic acid, butyric acid, caprylic acid, cinnamic acid, citramalic acid, citric acid, enanthic acid, fumaric acid, galacturonic acid, gallic acid, glutaric acid, glycolic acid, glyoxylic acid, p-hydroxybenzoic acid, isocitric acid, α-ketoglutaric acid, lactic acid, malic acid, mandelic acid, phenylacetic acid, propionic acid, protocatechuic acid, pyruvic acid, salicylic acid, sorbic acid, succinic acid, tartaric acid, valeric acid, vanillic acid

KEY WORDS
derivatization

REFERENCE
Mentasti,E.; Gennaro,M.C.; Sarzanini,C.; Baiocchi,C.; Savigliano,M. Derivatization, identification and separation of carboxylic acids in wines and beverages by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *322*, 177–189.

Aspartame



Molecular formula: $C_{14}H_{18}N_2O_5$
Molecular weight: 294.31
CAS Registry No.: 22839-47-0
Merck Index: 874

SAMPLE
Matrix: beverages
Sample preparation: Filter sample.

HPLC VARIABLES
Column: 150 × 4.5 5 μm Hiasil C18 (Higgins)
Mobile phase: MeOH:25 mM phosphate buffer 45:55, pH 3.0
Flow rate: 1.0
Injection volume: 20
Detector: UV 218

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 1.4 mg/mL

OTHER SUBSTANCES
Extracted: benzoic acid, caffeine

KEY WORDS
comparison with UV spectrophotometry and capillary electrophoresis; soft drinks

REFERENCE
McDevitt,V.L.; Rodriguez,A.; Williams,K.R. Analysis of soft drinks: UV spectrophotometry, liquid chromatography, and capillary electrophoresis, *J.Chem.Educ.*, **1998**, *75*, 625–629.

SAMPLE
Matrix: beverages, formulations
Sample preparation: Dilute beverages and formulations with water, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb Hexyl
Mobile phase: MeOH:0.1% perchloric acid 15:85, pH 2.8
Flow rate: 1
Detector: E, ESA Coulochem Model 5100A, first electrode (screen mode) +0.10 V, second electrode (measuring electrode) +0.80 V, following post-column reaction. The column effluent flowed through a 20 m × 0.3 mm ID PTFE coil irradiated with a UV 254 lamp to the detector.

CHROMATOGRAM
Retention time: 13
Limit of detection: 500 ng/mL
Limit of quantitation: 1 μg/mL

OTHER SUBSTANCES
Simultaneous: caffeine

KEY WORDS
post-column reaction; soft drinks; post-column photochemical derivatization

REFERENCE
Galletti,G.C.; Bocchini,P. High-performance liquid chromatography with electrochemical detection of aspartame with a post-column photochemical reactor, *J.Chromatogr.A*, **1996**, *729*, 393–398.

SAMPLE
Matrix: beverages, sweetener
Sample preparation: Sweetener. Dissolve 30 mg powdered tabletop sweetener in water and dilute to 25 mL, filter (0.2 μm PTFE). Beverages. Dilute fruit juice 1:10 with water. Degas carbonated beverages in a ultrasonic bath for 5 min, dilute 1:10 with water, filter. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 50 × 4 Dionex IonPak AG4A-SC
Column: 250 × 4 Dionex IonPak AS4A-SC
Mobile phase: Gradient. A was 1 mM sodium carbonate. B was 12.5 mM sodium carbonate. A:B 100:0 for 4.5 min, from 100:0 to 0:100 over 1 min, maintain at 0:100 for 22.5 min, from 0:100 to 100:0 over 0.1 min
Flow rate: 1
Injection volume: 50
Detector: UV 190 for 6 min, UV 206 22 min, then UV 190; Conductivity, Dionex ED40 in conductivity mode preceded by a Dionex ASRS-I suppressor (external water mode, 300 mA)

CHROMATOGRAM
Retention time: 2.5
Limit of detection: 35 ng/mL (UV)

OTHER SUBSTANCES
Simultaneous: acesulfame, saccharin

REFERENCE
Chen,Q.-C.; Mou,S.-F.; Liu,K.-R.; Yang,Z.-Y.; Ni,Z.-M. Separation and determination of four artificial sweeteners and citric acid by high-performance liquid chromatography, *J.Chromatogr.A*, **1997**, *771*, 135–143.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30

Detector: UV 200.5

CHROMATOGRAM

Retention time: 9.8

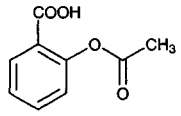
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Aspirin



Molecular formula: $C_9H_8O_4$
Molecular weight: 180.16
CAS Registry No.: 50-78-2
Merck Index: 886

SAMPLE

Matrix: blood
Sample preparation: Add 250 μL 200 mM orthophosphoric acid to 250 μL chilled plasma within 10 min of centrifuging (if fresh plasma) or within 10 min of thawing (if frozen plasma), vortex for 20 s, centrifuge at 5800 g for 3 min. Inject a 200 μL aliquot onto column A and elute to waste with mobile phase A, after 2 min backflush the contents of column A onto column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B.

HPLC VARIABLES

Column: A 10 × 4.3 30 μm Hypersil C18 PEEK cartridge (Shandon, England); B 10 × 4 30 μm Hypersil C8 + 250 × 4.6 5 μm Nucleosil C8
Mobile phase: A Water:orthophosphoric acid 1000:1, pH 2.5; B MeCN:MeOH:water:orthophosphoric acid 150:200:650:1 (pH 2.6)
Flow rate: 1
Injection volume: 200
Detector: UV 225

CHROMATOGRAM

Retention time: 33
Limit of detection: 40 ng/mL
Limit of quantitation: 100 ng/mL

OTHER SUBSTANCES

Extracted: salicylic acid
Noninterfering: barbital, butobarbital, caffeine, 8-chlorotheophylline, clonazepam, cocaine, diazepam, flurazepam, furosemide, hydralazine, imipramine, nitrazepam, phenytoin, pindolol, propranolol, quinidine, theophylline
Interfering: xylazine, prazosin

KEY WORDS

column-switching; plasma

REFERENCE

McMahon,G.P.; Kelly,M.T. Determination of aspirin and salicylic acid in human plasma by column-switching liquid chromatography using on-line solid-phase extraction, *Anal.Chem.*, **1998**, *70*, 409–414.

SAMPLE

Matrix: formulation
Sample preparation: Weigh 500 mg homogenized analgesic powder, transfer to 100 mL volumetric flask, add ca. 50 mL mobile phase, swirl and dilute to volume with mobile phase. Dilute an aliquot of this solution 1:10 with mobile phase, filter (0.20 μm Cameo nylon filter, MSI, Westboro, MA) an aliquot, inject an aliquot of the filtrate.

HPLC VARIABLES

Column: 100 × 2.1 5 μm Hypersil ODS

Mobile phase: MeCN:triethylamine:acetic acid:water 5.5:0.2:0.2:94.1 (Prepare mobile phase as follows. Mix 110 mL MeCN, 4 mL triethylamine, 4 mL glacial acetic acid and make up to 2 L with water.)
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Extracted: acetaminophen, caffeine
Interfering: salicylic acid

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Add 50 mL of mobile phase to 0.5 g of sample and swirl to aid dissolution. Dilute to 100 mL with mobile phase. Dilute 1:10, filter (0.22 μm nylon). Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 2.1 5 μm Hypersil ODS
Mobile phase: MeCN:water:triethylamine:acetic acid 5.5:94.1:0.2:0.2
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 1.4

OTHER SUBSTANCES
Simultaneous: acetaminophen, caffeine

KEY WORDS
powder

REFERENCE
Ferguson,G.K. Quantitative HPLC analysis of an analgesic/caffeine formulation: Determination of caffeine, *J.Chem.Educ.*, **1998**, *75*, 467–469.

SAMPLE
Matrix: formulations
Sample preparation: Sonicate 75 mg powdered tablets with 25 mL mobile phase for 15 min, filter (paper), inject a 135 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrabase C18 Scharlau Science, Spain
Mobile phase: MeOH:20 mM pH 4.KH_2PO_4 30:70 (pH adjusted with orthophosphoric acid)
Flow rate: 1.5
Injection volume: 135

Detector: UV 224

CHROMATOGRAM
Retention time: 12.8
Limit of quantitation: 3.6 μg/mL

OTHER SUBSTANCES
Simultaneous: caffeine, salicylic acid, thiamine

KEY WORDS
tablets

REFERENCE
Gámiz-Gracia,L.; Luque de Castro,M.D. An HPLC method for the determination of vitamin B1, caffeine, acetylsalicylic acid, and the impurities of salicylic acid in a pharmaceutical preparation, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2123–2133.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out powdered sample containing 68 mg aspirin, add 80 mL MeOH, sonicate for 10 min, dilute to 100 mL with MeOH, centrifuge. Remove a 5 mL aliquot of the supernatant and add it to 1 mL 2 mg/mL resorcinol, add 2 mL MeOH, make up to 20 mL with 50 mM pH 3.0 triethylamine phosphate, inject an aliquot.

HPLC VARIABLES
Column: 150 × 3.2 5 μm Hypersil ODS
Mobile phase: THF:50 mM pH 3.0 triethylamine phosphate 12:88
Flow rate: 0.6
Injection volume: 20
Detector: UV 275 following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID crocheted PTFE coil irradiated with an 8 W low-pressure mercury lamp at 254 nm to the detector.

CHROMATOGRAM
Retention time: 15
Internal standard: resorcinol (9)

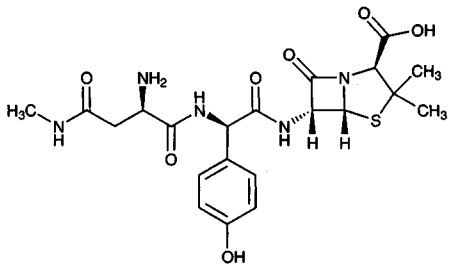
OTHER SUBSTANCES
Simultaneous: acetaminophen (post-column irradiation gives little increase in peak height), caffeine (post-column irradiation gives little increase in peak height), propyphenazone (post-column irradiation gives a decrease in peak height)

KEY WORDS
post-column reaction; post-column photochemical derivatization

REFERENCE
Di Pietra,A.M.; Gatti,R.; Andrisano,V.; Cavrini,V. Application of high-performance liquid chromatography with diode-array detection and on-line post-column photochemical derivatization to the determination of analgesics, *J.Chromatogr.A*, **1996**, *729*, 355–361.

Aspoxicillin

Molecular formula: $C_{21}H_{27}N_5O_7S$
Molecular weight: 493.54
CAS Registry No.: 63358-49-6
Merck Index: 887



SAMPLE

Matrix: blood, tissue, urine

Sample preparation: Serum, plasma. Dilute serum or plasma 1:2 to 1:10 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Urine. Dilute urine 1:10 to 1:100 with buffer, centrifuge, inject a 20 μL aliquot of supernatant. Tissue (lung, gut). Cut tissue with a scalpel, homogenize with 1-3 mL buffer, centrifuge at 9600 g for 5 min three times, inject a 20 μL aliquot. Tissue (chondral). Cut tissue with a scalpel, homogenize with 3-6 mL buffer in an ice bath for 2-3 min, centrifuge at 9600 g for 5 min four or five times, inject a 100 μL aliquot. Dilute human pleural samples with buffer, centrifuge, inject a 20 μL aliquot. (Buffer was 66.6 mM K_2HPO_4 adjusted to pH 7.40 with KH_2PO_4.)

HPLC VARIABLES

Column: 200 × 4 5 μm Nucleosil C18

Mobile phase: MeOH:buffer 8:92, adjusted to pH 5.8 with phosphoric acid (Buffer was 57.4 mM K_2HPO_4 adjusted to pH 5.8 with phosphoric acid.)

Flow rate: 1

Injection volume: 20-100

Detector: UV 220

CHROMATOGRAM

Retention time: 12

Limit of detection: 500 ng/mL

KEY WORDS

serum; plasma; lung; gut; pleural; chondral

REFERENCE

Knöller,J.; König,W.; Schönfeld,W.; Bremm,K.D.; Köller,M. Application of high-performance liquid chromatography of some antibiotics in clinical microbiology, *J.Chromatogr.*, **1988**, *427*, 257–267.

SAMPLE

Matrix: broncho-alveolar lavage fluid

Sample preparation: 1 mL Broncho-alveolar lavage fluid + 100 μL 5 μg/mL amoxicillin, vortex for 10 s, filter (Tosoh Ultracent-30 with a molecular mass cut-off at 30000) while centrifuging at 1500 g at 5° for 30 min, inject a 100 μL aliquot of the ultrafiltrate.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Shodex C18 5A (Showa Denko)

Mobile phase: MeCN:50 mM pH 3.0 potassium hydrogen phosphate containing 20 mM sodium 1-heptanesulfonate and 5 mg/L EDTA 10:100

Column temperature: 40

Flow rate: 1.2

Injection volume: 100

Detector: E, Irica Σ875, glassy carbon electrode 800 mV, Ag/AgCl reference electrode, following 10 m × 0.3 mm PTFE tubing irradiated by a GL-10 10 W mercury lamp

CHROMATOGRAM

Retention time: 24
Internal standard: amoxicillin (17)
Limit of detection: 1 ng/mL

KEY WORDS

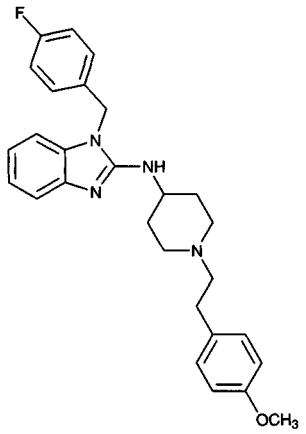
ultrafiltrate

REFERENCE

Yamazaki,T.; Ishikawa,T.; Nakai,H.; Miyai,M.; Tsubota,T.; Asano,K. Determination of aspoxicillin in broncho-alveolar lavage fluid by high-performance liquid chromatography with photolysis and electrochemical detection, *J.Chromatogr.*, **1993**, *615*, 180–185.

Astemizole

Molecular formula: $C_{28}H_{31}FN_4O$
Molecular weight: 458.58
CAS Registry No.: 68844-77-9
Merck Index: 891
Lednicer No.: 3 177



SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.16

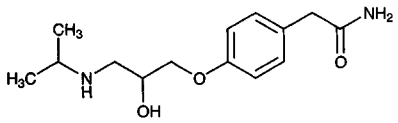
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Atenolol



Molecular formula: $C_{14}H_{22}N_2O_3$
Molecular weight: 266.34
CAS Registry No.: 29122-68-7
Merck Index: 892
Lednicer No.: 2 109

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 250 ng albuterol, mix for 10 s, add 10 mL dichloromethane:2-propanol 75:25, shake for 10 min. Centrifuge at 2000 g for 10 min at 4°. Remove the organic phase and evaporate it to dryness under a stream of nitrogen at 50°. Reconstitute the residue in 200 μL mobile phase , mix for 10 s. Centrifuge at 6500 g for 10 min. Inject a 40 μL aliquot of the supernatant.

HPLC VARIABLES

Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm Supelcosil LC-18 (Supelco)
Mobile phase: n-Propanol:buffer 5:95 (Buffer was 50 mM sodium dodecyl sulfate in 10 mM pH 5.8 sodium phosphate buffer.)
Flow rate: 1.3
Injection volume: 40
Detector: F ex 222 em 300

CHROMATOGRAM

Retention time: 15.7
Internal standard: albuterol (20.6)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES

Noninterfering: chlorthalidone, xipamide

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Giachetti,C.; Tenconi,A.; Canali,S.; Zanolo,G. Simultaneous determination of atenolol and chlorthalidone in plasma by high-performance liquid chromatography. Application to pharmacokinetic studies in man, *J.Chromatogr.B*, **1997**, *698*, 187–194.

SAMPLE

Matrix: blood
Sample preparation: Centrifuge plasma or serum at 11 300 g for 7 min, inject a 200 μL aliquot onto column A, elute to waste with mobile phase A, after 6 min backflush the contents of column A onto column B with mobile phase B, after 6 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. (Reequilibrate column A with mobile phase A for 4 min.)

HPLC VARIABLES

Column: A 20 × 4.0 BioTrap 500 C18 (ChromTech); B 12.5 × 4.6 5 μm Zorbax SB-CN + 150 × 4.6 5 μm Zorbax SB-CN
Mobile phase: A 2-Propanol:30 mM pH 7.0 sodium phosphate buffer containing 5 mM sodium octanesulfonic acid 2:98; B MeCN:30 mM pH 3.0 sodium phosphate buffer containing 2 mM sodium octanesulfonic acid 25:75

Flow rate: A 0.8; B 1
Injection volume: 200
Detector: F ex 230 em 300

CHROMATOGRAM
Retention time: 9.5

KEY WORDS
plasma; serum; column-switching

REFERENCE
Hermansson,J.; Grahn,A.; Hermansson,I. Direct injection of large volumes of plasma/serum on a new biocompatible extraction column for the determination of atenolol, propranolol and ibuprofen. Mechanisms for the improvement of chromatographic performance, *J.Chromatogr.A*, **1998**, *797*, 251–263.

SAMPLE
Matrix: blood, tissue
Sample preparation: Plasma, whole blood. Condition a Bond-Elut C8 SPE cartridge with MeOH and water. Mix plasma or whole blood and 50 mM pH 9 borate buffer, add to the SPE cartridge. Wash with water and MeCN. Elute with MeOH. Evaporate the eluate to dryness and reconstitute in 400 μL mobile phase. Inject a 50μL aliquot. Tissue. Homogenize (Braun micro-dismembrator) 100 mg tissue with 400 μL water while frozen in liquid nitrogen, thaw, rinse twice with 250 μL 1 M pH 3 potassium phosphate buffer. Centrifuge at 2740 g at 20° for 20 min, separate supernatant (S1). Extract pellet with 1 mL MeOH for 15 min with sonication. Centrifuge at 20° for 10 min, evaporate the supernatant to dryness under a stream of nitrogen at 40°. Reconstitute residue in 500 μL 15 mM pH 3 potassium phosphate buffer add to S1, centrifuge. Suck sample slowly through a Bond-Elut C8 SPE cartridge. Wash twice with water, elute twice with 200 μL MeOH, evaporate the eluate to dryness under a stream of nitrogen at 40°. Reconstitute in 500 μL mobile phase. Inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 17 × 4 5 μm Spherisorb C6
Column: 150 × 4.6 5 μm Spherisorb C6
Mobile phase: MeCN:15 mM pH 3 potassium phosphate buffer 17:83 (plasma) or 10:90 (tissue, blood)
Flow rate: 1
Injection volume: 50
Detector: UV 230

CHROMATOGRAM
Retention time: 4.4 (plasma), 6.8 (whole blood, tissue)
Internal standard: atenolol

OTHER SUBSTANCES
Extracted: sotalol

KEY WORDS
whole blood; plasma; heart; SPE; atenolol is IS

REFERENCE
Laer,S.; Neumann,J.; Scholz,H.; Uebeler,P.; Zimmermann,N. Determination of sotalol in human cardiac tissue by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *681*, 291–298.

SAMPLE
Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 3.637

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 10 μmole compound (as free base or hydrochloride) in 500 μL MeCN, add 250 μL 5% sodium carbonate (for hydrochlorides only), add 500 μL 100 mM reagent in MeCN, vortex for 1 min, heat at 60° for 2 h, add 100 μmole L-proline, heat at 60° for 30 min. Remove a 100 μL aliquot and dilute it with mobile phase, neutralize with acetic acid, inject a 10 μL aliquot. Prepare the reagent ((R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate) as follows. Add 0.7 mL carbon disulfide to 6 mL (1R,2R)-(-)-1,2-diaminocyclohexane, 12 mL water, and 12 mL EtOH, heat the oil bath to 80°, add 2.8 mL carbon disulfide dropwise (making sure that the product does not start to precipitate), when addition is complete reflux for 1 h, acidify with 500 μL 5 M HCl, reflux for 12 h, cool, filter, wash the solid with a little cold EtOH to give trans-4,5-tetramethyleneimidazolidine-2-thione as a white fluffy solid (mp 148-150°) (Tetrahedron 1993, 49, 4419). Stir 7.97 g 3,5-dinitrobenzoyl chloride in 30 mL dichloroethane at 50°, add a solution of 6 g trans-4,5-tetramethyleneimidazolidine-2-thione in 120 mL dichloroethane containing a catalytic amount of 4-(dimethylamino)pyridine over 15 min, reflux for 2 h, remove the crystals of (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate by filtration, evaporate the filtrate to dryness and dissolve the residue in 60 mL dichloroethane, reflux for 16 h to obtain more (R,R)-N-(3,5-dinitrobenzoyl)-2-aminocyclohexylisothiocyanate (mp >250°, $[\alpha]_{546}$ = -133° (c = 1) in MeCN).

HPLC VARIABLES
Column: 125 × 4 5 μm Lichrospher 60 RP Select B
Mobile phase: MeCN:20 mM ammonium acetate 55:45
Flow rate: 1

Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: k' 1.63, k' 2.24 (enantiomers)

OTHER SUBSTANCES
Also analyzed: acebutolol, alprenolol, carazolol, carvedilol, formoterol, methamphetamine, metipranolol, metoprolol, nifenanol, nitrilo atenolol, oxprenolol, pindolol, propranolol, xamoterol

KEY WORDS
derivatization; chiral

REFERENCE
Kleidernigg,O.P.; Posch,K.; Lindner,W. Synthesis and application of a new isothiocyanate as a chiral derivatizing agent for the indirect resolution of chiral amino alcohols and amines, *J.Chromatogr.A*, **1996**, *729*, 33–42.

SAMPLE
Matrix: perfusate
Sample preparation: Dilute perfusate 101 times with water and inject a 10 μL aliquot.

HPLC VARIABLES
Column: μBondapak C18
Mobile phase: MeCN:water:acetic acid 19:80:1 containing 625 nM 1-heptane-sulfonic acid
Flow rate: 2
Injection volume: 10
Detector: F ex 225 em300

CHROMATOGRAM
Retention time: 2.1
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: metoprolol

KEY WORDS
rat

REFERENCE
Lindahl,A.; Krondahl,E.; Grudén,A.-C.; Ungell,A.-L.; Lennernäs,H. Is the jejunal permeability in rats age-dependent?, *Pharm.Res.*, **1997**, *14*, 1278–1281.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:10 mM pH 3.5 sodium phosphate buffer 15:85
Flow rate: 1
Detector: UV 201

REFERENCE
Walter,E.; Janich,S.; Roessler,B.J.; Hilfinger,J.M.; Amidon,G.L. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: In vitro-in vivo correlation with permeability data from rats and humans, *J.Pharm.Sci.*, **1996**, *85*, 1070–1076.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: Dynamax C18 (Rainin)
Column: 250 × 4.6 5 μm Dynamax 300AC18 (Rainin)
Mobile phase: Gradient. A was MeCN:water:trifluoroacetic acid 10:90:0.1. B was MeCN:water:trifluoracetic acid 80:20:0.1. A:B from 100:0 to 70:30 over 12 min
Flow rate: 1
Detector: F ex 275 em 295

OTHER SUBSTANCES
Simultaneous: Tyr-containing peptides (F ex 278 em 305)

REFERENCE
Sorensen,M.; Steenberg,B.; Knipp,G.T.; Wang,W.; Steffansen,B.; Frokjaer,S.; Borchardt,R.T. The effect of β-turn structure on the permeation of peptides across monolayers of bovine brain microvessel endothelial cells, *Pharm.Res.*, **1997**, *14*, 1341–1348.

SAMPLE
Matrix: solutions
Sample preparation: Mix 300 μL of a 30 μM solution in dichloromethane with 10 μL 20 mM 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate in anhydrous dichloromethane and 50 μL 0.1% triethylamine in dichloromethane, vortex thoroughly, heat at 50° for 1.5 h, inject an aliquot. (Synthesize 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as follows (protect from light). Dissolve 500 mg (S)-(+)-naproxen in 50 mL dry toluene, slowly add 5 mL freshly distilled thionyl chloride, reflux for 1 h, evaporate to dryness under vacuum, dry the acyl chloride (mp 87.5°) under vacuum over KOH for 2 days. Dissolve 0.5 mmoles acyl chloride in 5 mL acetone, stir at 0°, add 0.6 mmoles sodium azide dissolved in ice water, stir at 0° for 30 min, add 10 mL ice-cold water, filter, dry solid in a desiccator under vacuum. Dissolve the solid in 1 mL toluene or dichloromethane (dried over 3 Å molecular sieve), reflux for 10 min, evaporate, store resulting isocyanate (mp 51°) under vacuum over a desiccant. Dissolve 0.5 mmole isocyanate in 5 mL acetone, add 20 mL 8.5% phosphoric acid, heat to 80° for 1.5 h, adjust to pH 13, extract with diethyl ether:dichloromethane 4:1. Wash the organic layer twice with water, dry over anhydrous sodium sulfate, evaporate to dryness, dissolve in 1 mL toluene, evaporate to give the amine from naproxen as crystals (mp 53°) (Pharm.Res. 1990, 7, 1262). Dissolve 1 mmole 1,1-thiocarbonyldiimidazole in 15 mL ice-cold chloroform, stir at 0°, add dropwise 1 mmole of the amine dissolved in 10 mL chloroform, stir at room temperature for 1.5 h, evaporate to dryness, reconstitute with carbon tetrachloride (Caution! Carbon tetrachloride is a carcinogen!), filter, evaporate the filtrate to dryness, store the resulting oil in a desiccator, purify on a short silica gel column with dichloromethane:light petroleum 50:50 to give 1-(6-methoxy-2-naphthyl)ethyl isothiocyanate as a slightly yellow liquid (store in the freezer under argon).)

HPLC VARIABLES
Column: 250 × 4 5 μm Zorbax ODS
Mobile phase: MeCN:water 50:50
Flow rate: 1
Injection volume: 100
Detector: UV 230, F ex 270 em 350

CHROMATOGRAM
Retention time: k' 5.2 (S-(-)), 6.1 (R-(+))

OTHER SUBSTANCES
Simultaneous: diacetolol

KEY WORDS
derivatization; chiral; F not much more sensitive than UV; $\alpha = 1.17$

REFERENCE
Büschges,R.; Linde,H.; Mutschler,E.; Spahn-Langguth,H. Chloroformates and isothiocyanates derived from 2-arylpropionic acids as chiral reagents: synthetic routes and chromatographic behaviour of the derivatives, *J.Chromatogr.A*, **1996**, *725*, 323–334.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 10 μm Partisil ODS1
Mobile phase: MeOH:50 mM pH 3.0 phosphoric acid 10:90
Column temperature: 30
Flow rate: 1.5
Detector: radioactivity detection

OTHER SUBSTANCES
Also analyzed: cimetidine, hydrochlorothiazide, ranitidine

KEY WORDS
tritium labeled

REFERENCE
Collett,A.; Sims,E.; Walker,D.; He,Y.-L.; Ayrton,J.; Rowland,M.; Warhurst,G. Comparison of HT29-18-C_1 and Caco-2 cell lines as models for studying intestinal paracellular drug absorption, *Pharm.Res.*, **1996**, *13*, 216–221.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 10 × 3.2 5 μm Partisil ODS3
Column: 100 × 4.6 5 μm Partisil ODS3
Mobile phase: MeCN:buffer 10:90 (Buffer was 60 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 0.6-1
Injection volume: 10-100
Detector: UV 270

OTHER SUBSTANCES
Also analyzed: practolol

REFERENCE
Palm,K.; Luthman,K.; Ungell,A.-L.; Strandlund,G.; Artursson,P. Correlation of drug absorption with molecular surface properties, *J.Pharm.Sci.*, **1996**, *85*, 32–39.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Vydac C18
Mobile phase: Gradient. A was 0.1% trifluoroacetic acid in water. B was 0.1% trifluoroacetic acid in MeCN. A:B from 95:5 to 65:35 over 9 min.
Column temperature: 40

Flow rate: 1
Detector: UV (wavelength not given)

OTHER SUBSTANCES

Simultaneous: dexamethasone

REFERENCE

Rubas,W.; Cromwell,M.E.M.; Shahrokh,Z.; Villagran,J.; Nguyen,T.-N.; Wellton,M.; Nguyen,T.-H.; Mrsny,R.J. Flux measurements across Caco-2 monolayers may predict transport in human large intestinal tissue, *J.Pharm.Sci.*, **1996**, *85*, 165–169.

SAMPLE

Matrix: solutions

Sample preparation: Mix a 100 μL of a 10 μM solution in MeCN:water:triethylamine 50:50:0.1 with 100 μL 1 mM (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in MeCN, heat in the dark at 65° for 1.5 h, inject an aliquot. (Synthesis of (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene from EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10° (use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ TLC plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei. Cool a solution of 16.4 g (S)-(-)-1-benzyl-3-pyrrolidinol in 164 mL pyridine to +5°, add 19.35 g p-toluenesulfonyl chloride, stir at +10° for 48 h, evaporate to dryness, chromatograph using dichloromethane:acetone 95:5 to obtain (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine (mp 68°). Heat a solution of (3S)-3-[(4-tolylsulfonyl)oxy]-1-(phenylmethyl)pyrrolidine in 200 mL anhydrous DMF to 65°, add 33.5 g sodium azide (Caution! Sodium azide is highly toxic!), stir at 60° for 7 h, filter, evaporate the filtrate to dryness under reduced pressure, dissolve the residue in ethyl acetate, wash twice with water, dry

over anhydrous magnesium sulfate, evaporate to obtain (3R)-3-azido-1-(phenylmethyl)pyrrolidine as an oil. Add 3.5 g 10% palladium on carbon under nitrogen to a solution of 7.05 g (3R)-3-azido-1-(phenylmethyl)pyrrolidine in 34.8 mL 1 M HCl in water and 245 mL EtOH, hydrogenate at atmospheric pressure for 30 min, add 3.5 g catalyst, hydrogenate for 2 h, filter, add 34.8 mL 1 M HCl to the filtrate, evaporate to dryness under reduced pressure, take up the residue in 70 mL EtOH, filter, evaporate the filtrate to dryness under reduced pressure, repeat this operation twice, crystallize with the minimum amount of EtOH to obtain (3R)-3-aminopyrrolidine dihydrochloride (J. Med. Chem. 1992, 35, 4205). 3R-(+)-aminopyrrolidine is also reported to be available from Tokyo Kasei. Add 100 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to a stirred solution of 200 mg 3R-(+)-aminopyrrolidine in 20 mL MeCN at 0-10°, stir at room temperature for 30 min, remove the MeCN by evaporation under reduced pressure, dissolve the residue in 50 mL 5% HCl, wash 3 times with 50 mL portions of ethyl acetate, adjust the pH of the aqueous solution to 13-14 with 5% NaOH, extract 6 times with 50 mL portions of ethyl acetate. Combine the organic layers and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane to obtain (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as orange crystals (mp 96-98°) (Analyst 1992, 117, 727). Add 100 μL thiophosgene in 10 mL benzene (Caution! Benzene is a carcinogen!) to 100 mg (R)-(-)-4-(3-aminopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole in 100 mL acetone, reflux for 1 h, remove the solvent by evaporation under reduced pressure, suspend the residue in 100 mL water, extract 4 times with 25 mL portions of benzene. Combine the extracts and wash them with 20 mL water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize from hexane:benzene 1:2 to obtain (R)-(-)-4-(3-isothiocyanatopyrrolidin-1-yl)-7-(N,N-dimethylaminosulfonyl)-2,1,3-benzoxadiazole as yellow crystals (mp 160-170° d) (Analyst 1995, 120, 385).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm Inertsil ODS-80A
Mobile phase: MeCN:water:trifluoroacetic acid 35:65:0.1
Column temperature: 40
Flow rate: 1
Detector: F ex 460 em 550

CHROMATOGRAM

Retention time: 26.7, 32.8 (enantiomers)
Limit of detection: 96-116 fmole

OTHER SUBSTANCES

Also analyzed: carteolol, timolol

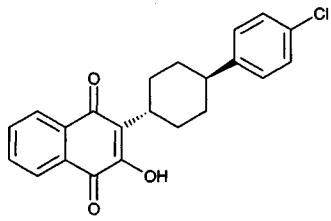
KEY WORDS

derivatization; chiral

REFERENCE

Toyo'oka,T.; Toriumi,M.; Ishii,Y. Enantioseparation of β-blockers labelled with a chiral fluorescent reagent, R(-)-DBD-PyNCS, by reversed-phase liquid chromatography, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 1467–1476.

Atovaquone



Molecular formula: $C_{22}H_{19}ClO_3$
Molecular weight: 366.84
CAS Registry No.: 95233-18-4
Merck Index: 898

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 1 mL phosphate buffer + 5 mL hexane:isoamyl alcohol 98:2, shake for 15 min, centrifuge at 1000 g for 5 min. Remove 2-4 mL organic layer and add it to 1 mL isopropanol, evaporate under a stream of nitrogen, dissolve in 200 μL MeOH:1% acetic acid 80:20, vortex for 10 s, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 4 Keystone C1 guard column
Column: 150 × 4.6 5 μm Supelcosil LC-1
Mobile phase: MeOH:1% acetic acid 70:30
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 7
Limit of quantitation: 250 ng/mL

OTHER SUBSTANCES

Simultaneous: pentamidine, zidovudine, trimethoprim, sulfamethoxazole

KEY WORDS

plasma

REFERENCE

DeAngelis,D.V.; Long,J.D.; Kanics,L.L.; Woolley,J.L. High-performance liquid chromatographic assay for the measurement of atovaquone in plasma, *J.Chromatogr.B*, **1994**, *652*, 211–219.

SAMPLE

Matrix: blood
Sample preparation: 100 μL Plasma + 1 mL 50 mM pH 7.0 KH_2PO_4/NaOH buffer + 5 mL hexane:3-methyl-1-butanol 98:2, tumble mix for 11 min, centrifuge at 1500 rpm for 11 min. Remove a 2 mL aliquot of the organic layer and add it to 800 μL isopropanol and 200 μL 100 ng/mL IS in isopropanol, evaporate under reduced pressure at 5 psi at 50° for 11 min, reconstitute with 500 μL hexane:3-methyl-1-butanol 98:2, evaporate for 11 min, reconstitute with 200 μL MeOH:1% pH 3.1 acetic acid 80:20, vortex for 15 s, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 reverse phase stainless steel column (Chrompack)
Column: two 100 × 3 5 μm Chromspher C8 glass columns in series (Chrompack)
Mobile phase: MeCN:0.4% pH 2.0 trifluoroacetic acid 65:35
Flow rate: 0.6
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 9

Internal standard: trans-2-hydroxy-3-(4-phenylcyclohexyl)-1,4-naphthalenedione (59C80) (7)
Limit of quantitation: 250 ng/mL

KEY WORDS
plasma

REFERENCE
Studenberg,S.D.; Long,J.D.; Woolf,J.H.; Bruner,C.J.; Wilson,D.; Woolley,J.L. A robotics-based liquid chromatographic assay for the measurement of atovaquone in plasma, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 1383–1393.

SAMPLE
Matrix: blood
Sample preparation: Heat plasma at 56° for 1 h. 200 μL Plasma + 50 μL 100 μg/mL IS in MeOH:DMF 99:1, mix well, add 400 μL MeCN:1% aqueous acetic acid 85:15, mix, centrifuge at 14000 g for 3 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb C6
Mobile phase: MeOH:0.2% pH 2 aqueous trifluoroacetic acid containing 10 mM triethylamine 76:24
Column temperature: 25
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 8.8
Internal standard: trans-2-hydroxy-3-(4-phenylcyclohexyl)-1,4-naphthalenedione (Burroughs-Wellcome) (7.4)
Limit of detection: 500 ng/mL

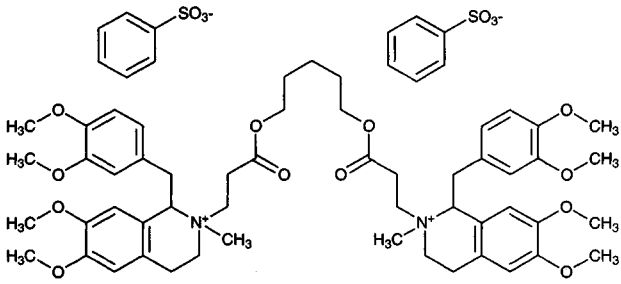
OTHER SUBSTANCES
Simultaneous: clindamycin, pentamidine, pyrimethamine, trimethoprim
Noninterfering: clarithromycin, dapsone, didanosine, fluconazole, folinic acid, foscarnet, gangiclovir, sulfadiazine, sulfamethoxazole, trimetrexate, zalcitabine, zidovudine

KEY WORDS
plasma

REFERENCE
Hansson,A.G.; Mitchell,S.; Jatlow,P.; Rainey,P.M. Rapid high-performance liquid chromatographic assay for atovaquone, *J.Chromatogr.B*, **1996**, *675*, 180–182.

Atracurium besylate

Molecular formula: $C_{65}H_{82}N_2O_{18}S_2$
Molecular weight: 1243.50
CAS Registry No.: 64228-81-5
Merck Index: 900



SAMPLE

Matrix: blood
Sample preparation: 250 µL Plasma + 250 µL picric acid (1:50 dilution of saturated picric acid solution) + 250 µL alcuronium solution + 250 µL water + 2.5 mL dichloromethane: isopropanol 85:15, vortex for 15 s, centrifuge at 1500 g for 10 min. Remove the organic phase and evaporate it to dryness at 40° under a stream of nitrogen, reconstitute the residue in 150-250 µL MeCN:water 40:60, centrifuge at 1500 g for 4 min, inject a 20-100 µL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 µPorasil
Mobile phase: MeCN:2 mM sulfuric acid 50:50
Flow rate: 2
Injection volume: 20-100
Detector: UV 210

CHROMATOGRAM

Retention time: 3.5
Internal standard: alcuronium (4.5)
Limit of detection: 25 ng/mL

OTHER SUBSTANCES

Also analyzed: tubocurarine, metocurine

KEY WORDS

plasma

REFERENCE

Bjorksten,A.R.; Beemer,G.H.; Crankshaw,D.P. Simple high-performance liquid chromatographic method for the analysis of the non-depolarizing neuromuscular blocking drugs in clinical anaesthesia, *J.Chromatogr.*, **1990**, *533*, 241–247.

SAMPLE

Matrix: blood
Sample preparation: Adjust pH of plasma to 4 with 2 M sulfuric acid. 250 µL Acidified plasma + 10 µL 0.5 M sulfuric acid + 50 µL 5 µg/mL verapamil in water, mix, add 600 µL MeCN, vortex for 1 min, centrifuge at 2400 g for 10 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 100 × 4.9 5 µm Spherisorb C8

Mobile phase: Gradient. A was MeCN:MeOH:30 mM K_2HPO_4 37.5:5:57.5, adjust final pH to 5. B was MeCN:MeOH:100 mM K_2HPO_4 37.5:15:47.5, adjust final pH to 5. A:B 0:100 to 100:0 over 8 min.
Flow rate: 1.7
Injection volume: 50
Detector: F ex 240 em 320

CHROMATOGRAM
Retention time: 6.2
Internal standard: verapamil (4)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Simultaneous: laudanosine

KEY WORDS
plasma

REFERENCE
Varin,F.; Ducharme,J.; Besner,J.G.; Théorêt,Y. Determination of atracurium and laudanosine in human plasma by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *529*, 319–327.

SAMPLE
Matrix: blood
Sample preparation: Condition a Waters C18 SPE cartridge with 4 mL MeOH and 8 mL water. 500 µL Plasma + 2 mL 15 mM sulfuric acid, add a 500 µL aliquot to the SPE cartridge, wash with 2 mL 5 mM PIC B-8, elute with 1.5 mL MeOH containing 5 mM PIC B-8, inject an aliquot of the eluate.

HPLC VARIABLES
Guard column: C-1 (Keystone)
Column: 150 × 4.6 Spherisorb C-1
Mobile phase: Gradient. A was water containing 5 mM octanesulfonic acid (PIC B-8). B was MeCN:water containing 5 mM octanesulfonic acid (PIC B-8). A:B 52:48 for 8 min, then A:B 12:88 for 9.5 min (step gradient), re-equilibrate at initial conditions for 4.5 min (step gradient).
Flow rate: 1
Detector: F ex 202 em 320

CHROMATOGRAM
Retention time: 15.5 (cis isomer)
Limit of detection: <40 nM

OTHER SUBSTANCES
Extracted: metabolites, laudanosine

KEY WORDS
human; rat; plasma; SPE

REFERENCE
Welch,R.M.; Brown,A.; Ravitch,J.; Dahl,R. The in vitro degradation of cisatracurium, the *R, cis-R'*-isomer of atracurium, in human and rat plasma, *Clin.Pharmacol.Ther.*, **1995**, *58*, 132–142.